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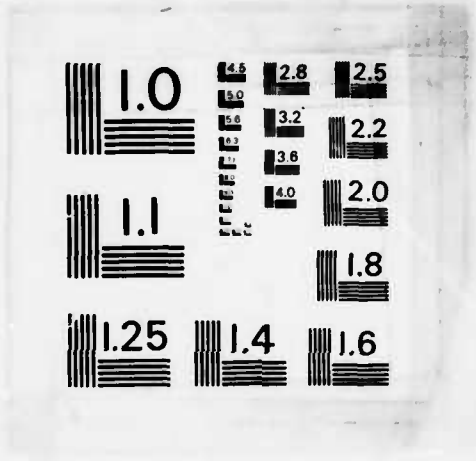
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Experimental methods have been developed and validated which will permit us to: 1) measure leakage of tissue enzymes (ALT, CPK, Alkaline phosphatase, LDH) during heat exposure in rats; 2) measure lithium concentrations in plasma using atomic absorption spectrophotometry; 3) measure total body water, extracellular fluid space, and intravascular fluid space in rats; 4) passively heat restrained rats and record changes in rectal temperature. Our objectives for future work include: 1) learn methods for study of unrestrained rats which are passively heated; 2) learn methods for study of rats actively heated using treadmill; 3) treat rats with lithium and determine whether passive restrained, unrestricted restrained, or actively heated lithium-treated rats can be differentiated from controls in terms of heating rate, tissue enzyme leakage, or body water distribution. KeyWords: Fatigue Physiology

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FOREWORD

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Introduction:

Our ultimate aim is to understand the factors which modulate susceptibility to heat-induced illness in humans. Only limited experimentation can be justified using healthy humans. Before we can initiate studies in humans we must confirm our hypothesis (that chronic lithium treatment predisposes to heat-induce illness) and finalize our techniques using an animal model. We believe that lithium-treated rats will develop heat-induced illness more easily and rapidly than will controls. Heat-induced illness is a serious medical emergency. Mortality rates of 17-70% are reported, and in the United States more than 4000 people per year die of heatstroke (1). Heat-associated decrements in performance, and heat-injury are serious problems for the military, as strenuous physical activity in hot climates must be required of soldiers in the field. Heat-induced illness is being seen with increased frequency in healthy civilian populations as jogging becomes more prevalent (2).

There are differences between those individuals who develop heatstroke and those who do not. Factors that have been thought to predispose to heat-induced illness include amount of exertion, prior conditioning, pre-existing cardiovascular disease, diabetes mellitus, impairment of sweating due to pharmacologic agents or sweat gland disease, potassium deficiency, and lithium treatment (1-7). Lithium administration results in altered distribution of body water, sodium depletion, potassium wasting, polyuria, and abnormal thermoregulation (2,3,8-17). Each of these effects may predispose to developing heat-induced illness, therefore a lithium-treated model of heat stroke will facilitate study of most of the factors believed to be integral to the development of these syndromes in humans.

The rat model of heat stroke

The high rate of mortality precludes the use of humans for controlled studies of the pathophysiology of heat stroke. Therefore suitable animal models are required. Hubbard and colleagues have worked extensively with rats and have found it to be a useful model (18, 19-25). Rats are small and inexpensive, and have been extensively studied for decades. We have a large foundation of information about the biology of the laboratory rat which allows us to make predictions about the cardiovascular, behavioral, and metabolic responses to heat stress. Finally, the rat is available in genetically controlled lines, making it possible to expect similar biological responses from different animals. This homogeneity does not exist for sheep or dogs (26,27).

The major mechanism for cooling in the rat is by an increase in blood flow to the tail (23). This is equivalent to the increase of skin blood flow in the human, and allows us to manipulate radiant heat loss (R). The major difference between the rat and the human is the mechanism used to mediate evaporative cooling. The rat does not sweat, but rather secretes copious amounts of saliva, with which it wets its surface and thereby loses heat by evaporation (E). Saliva production by the submaxillary gland is proportional to ambient temperature and core body temperature (23). This cooling mechanism involves behavioral and physiological functions which are different from those involved in sweating (23). This is a problem common to all animal models. Dogs, sheep, ferrets and cats cool themselves via panting. Evaporation during panting cools blood vessels feeding the brain, thereby lowering brain temperature. This mechanism differentially changes brain versus core temperature, and is very different than that which occurs in man. The licking and wetting cooling mechanism of the rat is far closer to the sweating that occurs in man. The average rate of water loss due to salivation and wetting in the rat is similar to the rate of sweat loss in unacclimatized man in heat (23).

Hubbard and colleagues (18, 19-25) have proved that a syndrome similar to human heatstroke can be induced in rats.

Lithium and thermoregulation

Lithium treatment alters thermoregulation in man and the rat. In lithium intoxication, body temperature is frequently markedly elevated, with temperatures

exceeding 40.8°C (2,3,11,28). Many of the symptoms of heat stroke are similar to those seen in lithium intoxication (3). In psychiatric patients, lithium treatment (not intoxication) results in consistently elevated body temperature throughout the 24 hr circadian temperature rhythm (11). In rats, lithium administration causes an acute hypothermia, but within 3 hours causes significant hyperthermia (body temperature increases of more than 1°C) (11,14). Lithium reverses the hypothermia associated with treating humans with electroshock therapy (28).

Lithium treatment makes humans more susceptible to heat stress. Individuals treated with lithium prior to marathon races have developed acute heat stroke (2). Rats treated with lithium for as little as 4 days develop fatal hyperpyrexia (core temperature increases more than 3°C) when treated with tranylcypromine, an MAO inhibitor. Tranylcypromine treatment alone, or in combination with pretreatment with sodium, potassium or rubidium was not associated with hyperpyrexia (12,29,30).

Based upon the above reports, lithium seems to act centrally to alter temperature setpoint, while at the same time it disturbs electrolyte and water distribution in the periphery. It may also increase metabolic production of heat because it activates the Na+/K+ ATPase pump. An understanding of each of these actions of lithium should help us to unravel the factors involved in heat production and dissipation, thereby increasing our understanding of the mechanisms causing heat stroke.

BODY:

Methods:

Animals: Male Sprague-Dawley rats, weighing 400 g, will be used in all experiments. They are caged individually in a room maintained at 26°C and 30% relative humidity and fed rat chow and water *ad libitum*.

Passively induced heat-illness: During heat testing, rats are housed in a small environmental chanber (modified tissue culture incubator) in individual containers. For some studies animlas are placed in restrainers which permit complete air circulation around the rat, but which restrict movement. Temperature in the chamber is maintained at 41.5° C with forced hot air, relative humidity at 30%. In control rats, this method of heating will elevate core temperature at 0.02 °C/min to a temperature of 42.3° C (18,19,20).

Exertion induced heat-illness: During heat-testing, animals are exercised on a motor-driven treadmill. It consists of a wide belt on metal rollers. One roller is driven at a selected rate by a variable speed motor. A lucite box, partitioned into individual compartments 30 cm long x 10 cm wide, is suspended over the belt, providing a separate running area for each rat. A shock grid is located at the rear of the compartments and the animals learn to avoid being shocked by keeping pace with the belt. The lucite chambers are heated with forced hot air to a temperature of 26° C. Humidity is maintained at 30% relative humidity. Animals are run at 5-11 m/min at a 0-6° incline until hyperthermic exhaustion (rectal temperature 42.3°C, animal unable to right itself). In control rats this method of heating will elevate core temperature by 0.15 °C/min (18,19,20).

Temperature measurement: Rectal temperature is monitored using a thermocouple placed 6.5 cm into the rectum.

Venous catheterization:

Method used has been published previously (31). Animals are anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Cannulas (Silastic tubing, .020 in. ID, .037 in. OD, Dow Corning) are inserted into the left carotid artery and the right jugular vein. To prevent slippage, a small amount of silicone adhesive (Medical Adhesive Silicone Type A, Dow Corning) is placed on the cannula at a distance relative to the size of the animal (the end of the cannulas should just reach the aortic arch and superior vena cava). A silk ligature is run through the silicone and tied to the vessel. The cannulas then may be exteriorized between the shoulder blades and flushed daily with heparinized saline (10 units/ml). Animals require 48 hr to recover from surgery .

Determination of Total Body Water, Extracellular Fluid Volume and Plasma Volume:

These methods are modifications of those previously described (32,33).

Place venous catheter in jugular vein, allow animal to recuperate 48 hours prior to use. Monitor body weight and core temperature, when both return to normal (48 hours), the rat is fit for experimentation. Each animal receives 50μCi of $^{3}H_2O$, 20μCi of $^{35}SO_4$ and 2μCi of ^{125}I-radioiodinated serum albumin. Prepare one solution so that 100 μl of 2% BSA contains all three isotopes. Inject the isotopes into the venous catheter with a Hamilton syringe. Record the time of injection. Flush the line with 300μl of heparinized saline (10 units/ml) and obstruct the line. Withdraw blood samples of 150μl from the venous catheter at 2, 4, 6, 30, 60 and 90 minutes post injection (before drawing blood, clear line of heparinized saline). Donor blood from rats treated in the same way as the study rats, is witdrawn and used to replace blood withdrawn from experimental animals. Then flush line with 30 μl of heparinized saline (10 units/ml)).

Spin blood to obtain plasma. Aliquot 50μl of each sample into a tube containing 0.5ml 2% BSA. Prepare iodine standard by adding 50μl of the ^{125}I-RISA injection

solution to a 10 ml volumetric flask. Bring to volume with water. Aliqout in triplicate 50µl of standard to a test tube containing 0.5 ml 2% BSA. DPM are determined using a gamma counter. To other 50 µl aliquots of plasma, add 1.0 ml of 10% TCA. Mix and centrifuge at 3000 rpm for 15 minutes (this precipitaes labelled albumin). Place 1.0 ml of supernatant in a 20 ml plastic scintillation vial and determine dpm for ^{3}H and ^{35}S using scintillation spectrophotometry. Prepare tritium and sulfur standard by adding 100µl of injection solution to 100 ml volumetric flask. Bring to volume with water. Count 1 ml of this after adding 60 µl of 10% TCA, using scintillation spectrophotometry.

Calculations: (The following calculations are from Fowler *et al.* (32), who use the separate isotope injection method. If the method is changed to a single injection, adjustments must be made in the standard preparation listed above in the constants described below.)

Total Body Water

$$(\text{TBW; ml}) = \frac{3.226\ (\text{dpm } {}^{3}H_2O_{adm}) \times ((100\text{-}CPl_{tp})/100)}{(\text{dpm plasma @ 0 min})}$$

where:
$^{3}H_2O_{adm}$ = administered $^{3}H_2O$ standard
CPl_{tp} = protein content of plasma in gram/100 ml
3.226 = constant correcting for dilution and aliquoting.

Extracellular fluid volume

$$(\text{ECF; ml}) = \frac{(0.9)\ (3.226)\ (\text{dpm } {}^{35}SO_{4\ adm}) \times ((100\text{-}CPl_{tp})/100)}{(\text{dpm plasma @ 0 min})}$$

where:
$^{35}SO_{4\ adm}$ = administered $^{35}SO_4$
CPl_{tp} = protein content of plasma in gram/100 ml
3.226 = constant correcting for dilution and aliquoting.
0.9 = constant for Donnan Equilibrium
dpm plasma @ 0 min = extrapolated from regression analysis of data.

Plasma Volume

$$(\text{PV; ml}) = \frac{10 \times (\text{dpm } RISA_{adm})}{(\text{dpm RISA @ 0 min})}$$

where:
$RISA_{adm}$ = administered ^{125}I-albumin
10 = constant correcting for dilution and aliquoting.
dpm RISA @ 0 min = extrapolated from regression analysis of data.

Measurement of Leakage of Tissue Enzymes into blood:

Alkaline Phosphatatase

We have set up and validated an assay for alkaline phosphatase activity in plasma (34) based upon the following enzymatic reactions.

p-nitrophenyl phosphate ----------> p-nitrophenol + P_i

p-nitrophenol + NaOH ------> colored complex

Add 50 µl each of 1.5M 2-Amino-2-methyl-1-propanol. pH 10.3 and p-nitrophenyl phosphate, disodium to a test tube and equilibrate to 37°C in a water bath. Pipet 10 µl serum to reaction mix and incubate 15 minutes at 37°C. Stop reaction by adding 1 ml of .05N NaOH. Record absorbance at 410 nm. Add 20 µl of concentrated HCl to remove color due to p-nitrophenol formation. Record absorbance at 410 nm due to serum color. Determine AP units by extrapolating from a calibration curve obtained from using diluted p-nitrophenol and 0.02N NaOH.

Alanine Aminotransferase (ALT)

Alanine aminotransferase (35) activity in plasma is measured based upon the following enzymatic reactions.

$$\text{L-Alanine + 2-Oxoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate + L-Glutamate}$$

$$\text{Pyruvate + NADH} \xrightarrow{\text{LDH}} \text{Lactate + NAD}$$

Prepare reaction mix consisting of 400mM L-Alanine, 12mM 2-Oxoglutarate, 2000 U/L Lactate Dehydrogenase and .25mM NADH, pH 7.4. Add 100 µl plasma to 1 ml reaction mix, incubate at 30°C for 90 seconds. Record absorbance at 340 nm. Incubate and record absorbance exactly 60 seconds after initial reading. Calculate ΔA per minute.

Creatine Phosphokinase (CPK)

CPK activity in plasma is measured (36) based upon the following enzymatic reactions.

$$\text{ATP + Creatine} \xrightarrow{\text{CPK}} \text{ADP + Phosphocreatine}$$

$$\text{Phosphocreatine} \xrightarrow{\text{hydrolysis}} \text{Creatine + Pi}$$

Pi + Acid Molybdate -----> Colored Complex

Mix 100 µl of 0.06M Creatine and $MgSO_4$ in TRIZMA Buffer, pH 9.0, 30µl serum and 100µl water. Run corresponding blank without .06M Creatine in the TRIZMA Buffer. Add 10 µl of .5mM ATP and reduced glutathione. Incubate 30 minutes at 37°C. Stop reaction by adding 160 µl cold 20% TCA. Centrifuge 5 minutes at 3000 rpm. Add 100 µl clear supernatant to 400 µl water, 100µl 1.25% Acid Molybdate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in 2.5 N sulfuric acid and 25 µl of 1-Amino-2-naphthol-4-sulfonic acid, 0.8% sodium sulfate and sodium bisulfite solution (1 gram mixed in 6.3 ml water). Incubate at room temperature for 30 minutes. Read absorbance at 660 nm. Calibrate against known amounts of inorganic phosphate. Relate Δ µgrams P_i to CPK activity.

Lactate Dehydrogenase (LDH)

LDH activity is measured in plasma (37) based upon the following enzymatic reactions.

$$\text{Pyruvic Acid} + \text{NADH} \xrightarrow{\text{LDH}} \text{Lactic Acid} + \text{NAD}$$

Remaining pyruvate + 2,4-dinitrophenylhydrazine ----> hydrazone

hydrazone + NaOH -----> colored complex

Equilibrate .75 mM Sodium Pyruvate solution, pH 7.5 with 1 mg NADH to 37°C in a water bath. Add 10 µl diluted serum (1 part serum to 5 parts water). Incubate for 30 minutes at 37°C. Add 100 µl of 2,4-dinitrophenylhydrazine, 20 mg/dL in 1N HCl, incubate 20 minutes at room temperature. Add 1 ml of .40M NaOH, incubate 5 minutes at room temperature. Read absorbance at 450 nm. Extrapolate against calibration curve prepared by adding known amounts of pyruvate to water and 2,4-dinitrophenylhydrazine, and relating absorbance to LDH activity.

Measurement of Plasma and Tissue Lithium Concentrations:

Plasma is diluted so final concentration is 10% plasma and 10% HCl (for example, add 500 µl plasma and 500 µl concentrated HCl to 5 ml water. Total lithium is measured by atomic absorption spectrophotometry using a Perkin Elmer Zeeman 5000 Atomic Spectroscopy System calibrated to a 1 ppm Lithium standard prepared by adding 50µl of 1000 ppm standard to 5 ml normal plasma, 5 ml concentrated HCl and 40 ml water.

RESULTS:

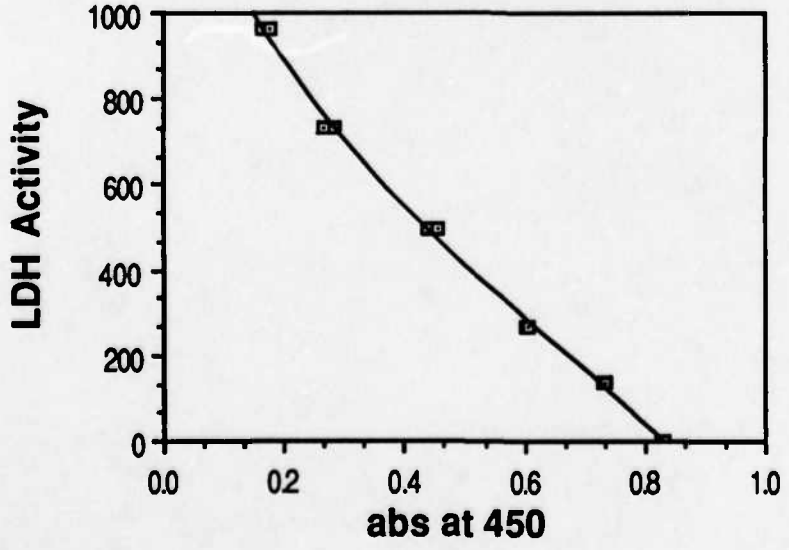
Methods Development:

LDH Assay

We have set up and validated an assay for LDH activity in plasma. We find that the results are reproducible. In 5 rats mean LDH activity was 192 ± 8.3 SEM. Published values in the Sprague Dawley rat (Charles River Professional Services Technical Bulletin) are 167 (range 130-204). Conclusion: Our measurement is consistent with published data.

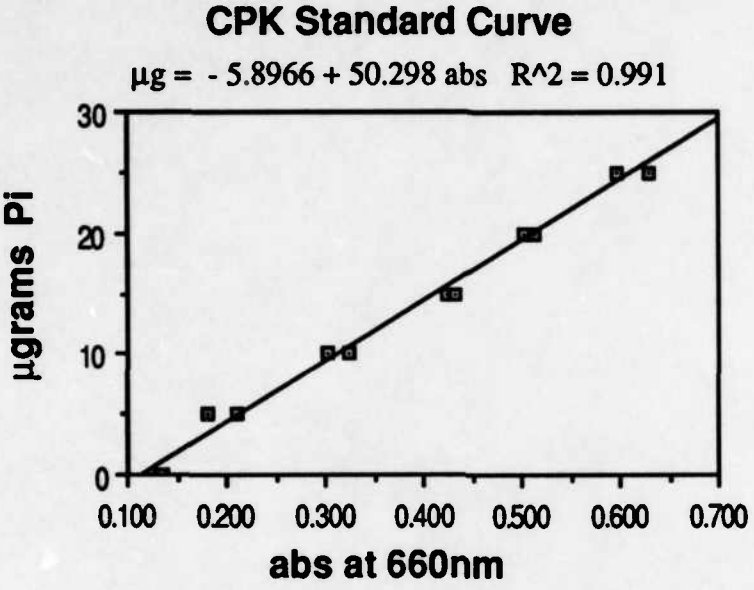
Lactate Dehydrogenase Standard Curve

LDH = 1386.5 - 2968.6 abs + 2761.9x^2 - 1442.1x^3 R^2 = 0.998

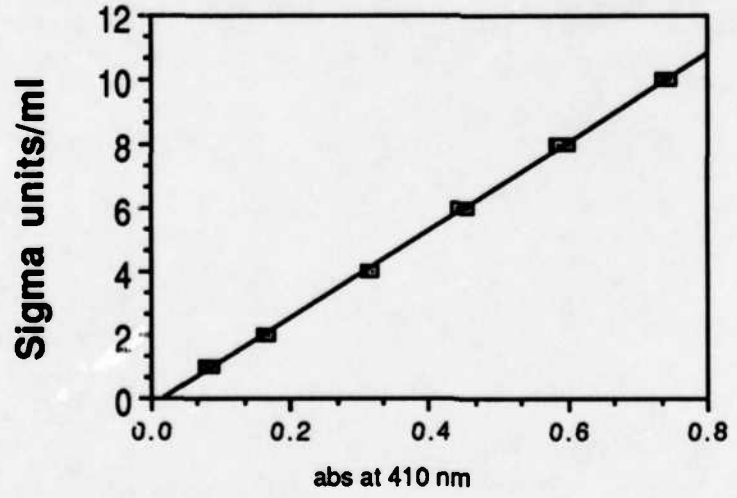


CPK Assay

We have set up and validated an assay for CPK activity in plasma. We find that the results are linear and reproducible. In 5 rats mean CPK activity was 203 ± 8.6 SEM. Published values in the Sprague Dawley rat (Charles River Professional Services Technical Bulletin) are 253 (range 202-308). Conclusion: Our measurement is consistent with published data.



ALKALINE PHOSPHATASE Assay:



AP Activity = - 0.22019 + 13.810 Abs R^2 = 0.999

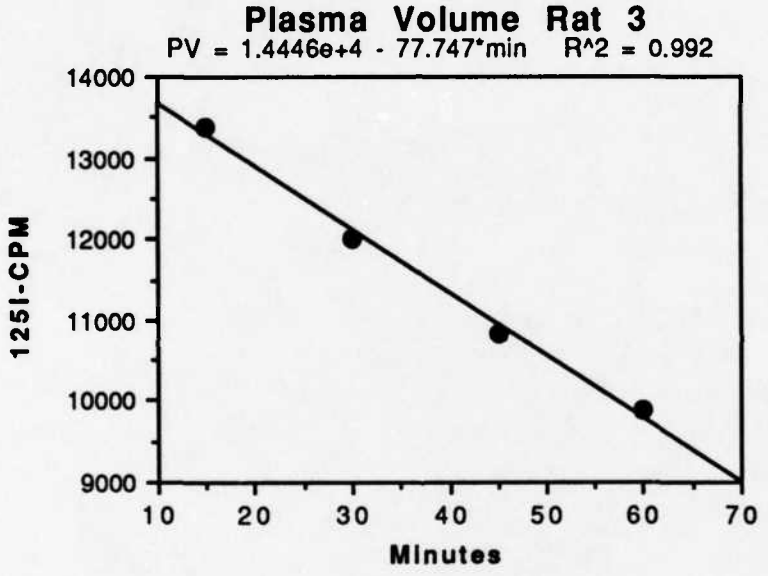
We have set up and validated an assay for alkaline phosphatase activity in plasma. We find that the results are linear and reproducible. In 5 rats mean Alk Phos activity was 206 ± 2 SEM. Published values in the Sprague Dawley rat (Charles River Professional Services

Technical Bulletin) are 405 (range 289-521). Conclusion: Our measurements are slighly lower than published values, but are probably reasonable in the rat.

ALT Assay:
We have set up and validated an assay for ALT activity in plasma. ALT values are calculated from change in absorbance, and we use normal human serum standards to calibrate the assay. We find that the results are reproducible. In 5 rats mean ALT activity was 104 ± 13 SEM. Published values in the Sprague Dawley rat (Charles River Professional Services Technical Bulletin) are 150 (range 76-224). Conclusion: Our measurement is consistent with published data.

BODY WATER POOLS:

TYPICAL ISOTOPE DISTRIBUTION DATA IN A RAT

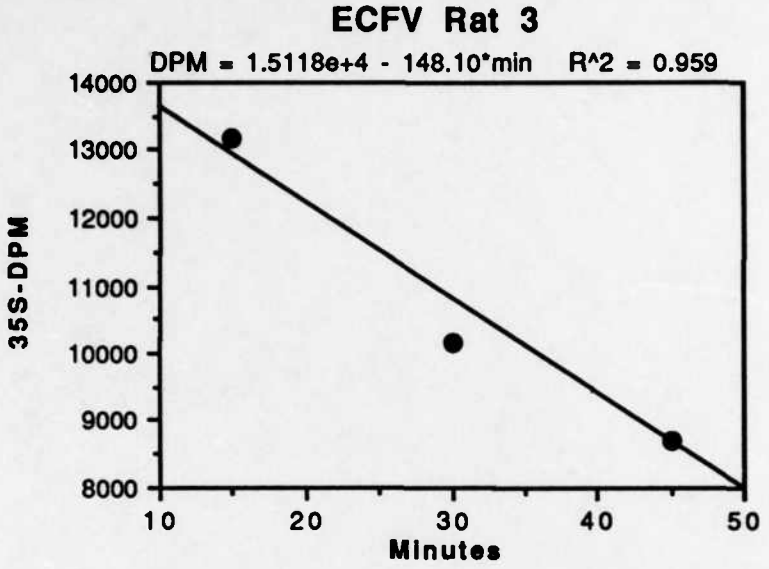


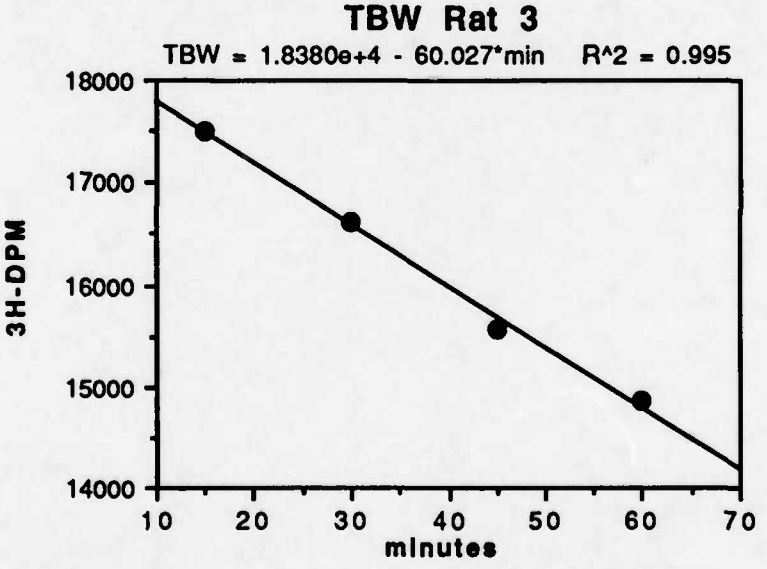
We have developed our measurements of body water compartments and have tested them in rats. In our first attempt using this method we generated results which did not agree well with published data (Exp. 1). We refined our techniques and repeated the determinations. Experiment 2 produced more reasonable values for body water distribution. However, we still underestimated total body water (TBW) and intracellular fluid (ICF), and overestimated extracellular volume (ECFV) and interstitial fluid (ISF).

Table: Comparison of body water distribution measurements in control rats

	Exp 1	Exp 2	Durkot *et. al.* (33)
TBW (%)	28.70±7.52	59.58±6.81	69.94±0.47
ECFV (%)	5.06±0.55	25.09±2.25	17.37±0.74
PV (%)	0.93±0.002	5.31±0.53	4.48±0.09
ICF (ml)	72.62±16.1	108.74±10.5	188.10±1.4
ISF (ml)	12.83± 1.5	65.28±15.8	44.20±2.5
Body weight (g)	315.8±32	327.7±60	300±20

Conclusion: Although our technique for measuring body water distribution has improved, there are still some methodologic problems. We believe that these problems are due to inadequate equilibration time allowed for $^{3}H_2O$ equilibration and to some diffusion into the cell of $^{35}SO_4$. These would account for the low and high TBW and ECFV results, respectively. For these reasons, we have decided to modify our techniques and to employ the methods of Durkot, *et. al.* (33). ^{14}C-inulin will replace $^{35}SO_4$ as the ECFV marker, and $^{3}H_2O$ will be allowed to equilibrate for at least 90 min. We believe that these changes will help to improve our results. We are currently testing these new methods.





Standards (dose injected):
^{125}I= 21,163 cpm

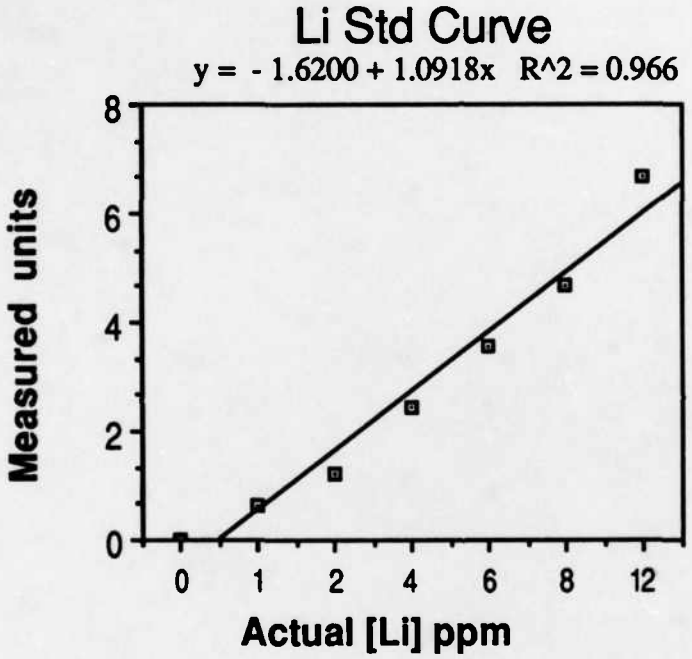
$^{35}SO_4$= 384,048 dpm
^{3}H= 1,034,968 dpm

Rat 3 body weight= 279 g
protein content of plasma/100ml=6.3 g

Substitution of this data into the equations on p. 8 gives values of 61.00% for TBW, 24.66% for ECFV and 5.25% for PV. In addition, the volumes for ICF and ISF are 101.4 and 54.2 ml, respectively.

Lithium Determination:

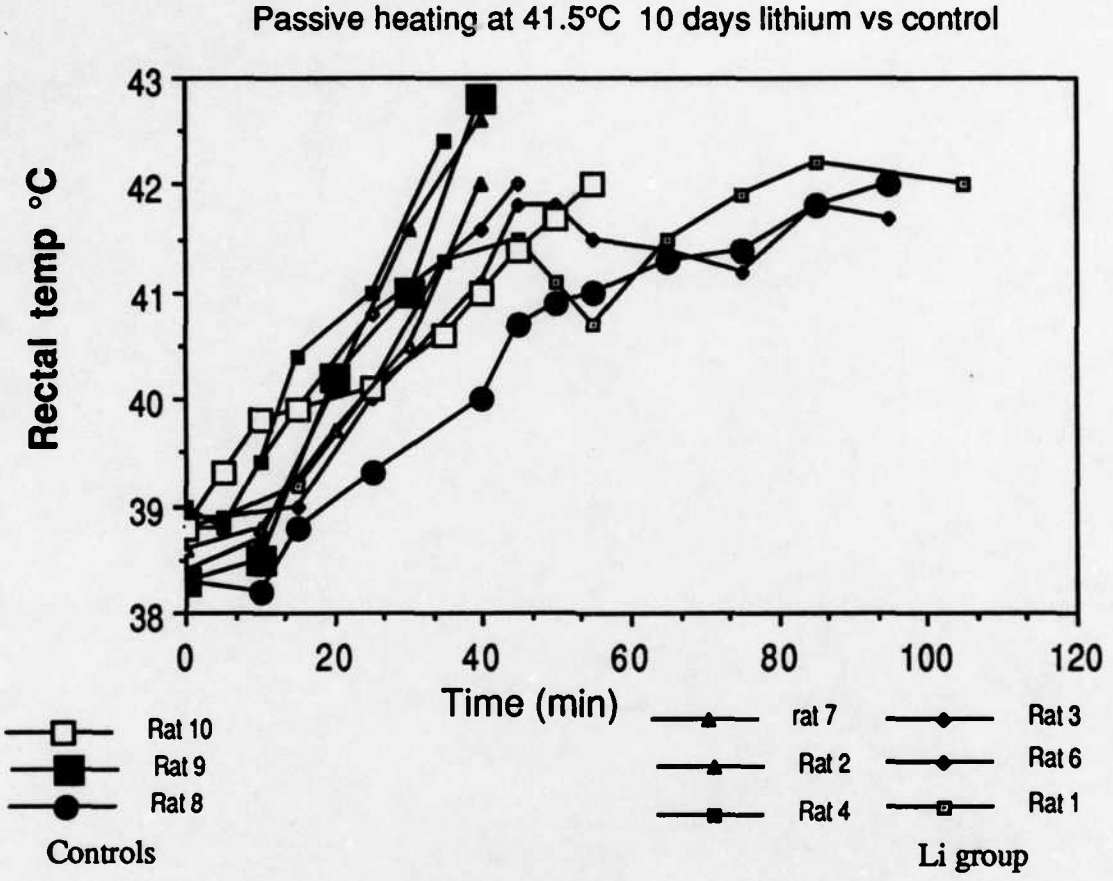
We have developed the Atomic Absorption spectrophotometric assay for lithium in plasma, and get reproducible results.



Conclusion: Lithium assay is usable as developed.

Passive Heating Procedures:

We have developed a small environmental chamber in which passive heating of rats can be performed. We performed pilot experiments using 10 rats, in which rats were treated with 2 MEq lithium/day (or with water) by orogastric intubation for 10 days. Animals were placed in the environmental chamber at 41.5°C and rectal temperature was measured at 5-10 minute intervals in unrestrained rats.



We found that rats heated up faster than expected, and after consultation with US ARIEM, Natick, conclude that rats were probably stressed by changes in housing, and handling. We also noted that frequent sampling of rectal temperature resulted in slight cooling of the environmental chamber (rats 1& 6). We plan to make the following changes in our procedure:

1) Rats will be handled daily, and have a rectal temperature taken daily so as to train them to reduce stress. This will also provide useful information as to baseline temperature regulation in lithium treated rats.

2) The environmental chamber will be housed in the animal facility so as to minimize transportation related stress to rats.

3) For an initial experiment, we will restrain rats so as to minimize variability, and to make it easier for us to take rectal temperature without cooling down the environmental chamber. This will also provide useful information as to non-salivation mediated cooling processes in the two treatment groups.

4) We will measure body water pools in different (but treatment-matched) animals so as to avoid surgical and blood drawing stresses.

CONCLUSIONS:

Most of our progress has been made in methods development.
We have developed:
ALT assay
CPK assay
LDH assay
Alkaline phosphatase assay
Lithium assay
Total Body Water determination
Extracellular Fluid Volume determination
Plasma Volume determination
Passive heating paradigm methods

The original Research technician working on the project left in September to go to graduate school, a replacement was hired and trained for 2 months, but was not able to perform the testing in a reliable manner, and has been replaced with a new technician who is again being trained. We have used these training periods to re-validate our techniques. We believe that most of the methods are now in place, and that with minor refinements we will be able to proceed with testing our hypotheses.

REFERENCES CITED

1. Clowes GHA, O'Donnell TF (1974) N. Engl. J. Med. 291:564-566.
2. Lowance D (1980) J. Med. Assoc. Georgia 69:284-286.
3. Granoff AL, Davis JM (1978) J. Clin. Psychiat. 39:103-107.
4. Editorial (1968) Lancet 2:31-32.
5. Shibolet S, Lancaster MC, Danon Y (1976) Av.Space Environ. Med. 47:280-301.
6. Greenleaf JE, Van Beaumont W, Brock PJ, Montgomery LD, Morse JT, Shvartz E, Kravik S (1980) Av.Space Environ. Med. 51:747-753.
7. Sarnquist F, Larson CP (1973) Anesthesiology 39:348-350.
8. Maletzky B, Blachly PH (1971) in: The Use of Lithium in Psychiatry , CRC Press, Cleveland Ohio. pp. 41-55.
9. DePaulo JR, Correa E, Sapir DG (1984) Biol. Psychiat. 19:1345-1349.
10. Shou M, Amdisen A, Trap-Jensen J (1972) in: Lithium in Medicine (J Mendels, SK Secunda, eds), Gordon & Breach Science Publishers, London. pp.112-119.
11. Mellerup ET, Widding A, Wildschiodtz G, Rafaelson OJ (1978) Acta Pharmacol. Toxicol. 42:125-129.
12. Shimomura K, Satoh Y, Mori J (1980) Japan J. Pharmacol. 30:268-271.
13. Martinez-Maldonado M, Stavroulaki-Tsapara A, Tsaparas N, Suki WN, Eknoyan G (1975). J. Lab. Clin. Med. 86:445-461.
14. Tulunay FC (1976) Pharmacol. 14:422-427.
15. Lee RV, Jampol LM, Browm WV (1971) N. Engl. J. Med. 284:93-94.
16. Abramov M, Cogan E (1984) Adv. Nephrol. 13:29-34.
17. Maickel RP, Tocco DR (1983) Res. Comm. Chem. Path. Pharmacol. 39:201-210, 1983.
18. Hubbard RW, Matthew WT, Criss REL, Kelly C, Sils I, Mager M, Bowers WD, Wolfe D (1978) J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 45:463-468.
19. Hubbard RW, Bowers WD, Matthew WT, Curtis FC, Criss REL, Sheldon GM, Ratteree JW (1977) J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 42:809-816.
20. Hubbard RW, Matthew WT, Linduska JD, Curtis FC, Bowers WD, Leav I, Mager M (1976) Am. J. Physiol. 231:1119-1123.
21. Hubbard RW, Criss REL, Elliott LP, Kelly C, Matthew WT, Bowers WD, Leav I, Mager M (1979) J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 46:334-339.
22. Francesconi R, Hubbard RW (1984) J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 57:733-738.
23. Hubbard RW, Matthew CB, Francesconi R (1982) J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 53:1171-1174.
24. Francesconi R, Hubbard RW (1985) J. Appl. Physiol. 58:152-156.
25. Francesconi R, Hubbard RW, Mager M (1983) J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 55:870-874.
26. Bell AW, Hales JRS, King RB, Fawcett AA (1983) J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 55:1916-1923.
27. Shapiro Y, Rosenthal T, Sohar E (1973) Arch. Int. Med. 131:688-692.
28. Lieb J, Lombard D, Nazzaro A (1980) Med. Hypoptheses 6:769-772.
29. Shimomura K, Hashimoto M, Mori J, Honda F (1979) Japan J. Pharmacol. 29:161-170.
30. Shimomura K, Tomoi M, Mori J (1979) Japan J. Pharmacol. 29:447-456.
31. Kelly, CB, Hubbard, RW, and Hamlet, M, A Method for the Chronic Cannulation of the Superior Vena Cava and the Aortic Arch in the Rat Using Cannulas Made of

Silicone Elastomer Rather than Polyethylene, U.S. Army Reseach Institute of Environmental Medicine, Report No. T 4/79, 1979.
32. Fowler, *et. al.*, Body fluid volumes in rats with menstranol-induced hypertension, *Am. J. Physiol.*, Vol. 25: July 1986, H190-195.
33. Durkot, *et. al.*, Simultaneous determination of fluid shifts during thermal stress in a small-animal model, *J. Appl. Physiol.* 61(3): 1031-1034, 1986.
34. Bessey O.A., Lowry O.H., and Brock M.J., A Method for the Rapid Determination of Alkaline Phosphatase with Fve Cubic Millileters of Serum, J. Biol. Chem., 164:321, 1946.
35. Bergmeyer H.U., Scheibe P. and Wahlefeld A.W., Optimization of Methods for Aspartate Aminotransferase and Alanine Aminotransferase, Clin. Chem., 24:58, 1978.
36. Okinaka S. *et al*, Serum creatine phosphokinase: Activity in progressive muscular dystrophy and neuromuscular diseases, Arch.Neurol., 4:520, 1961
37. Cabaud P. G., Wroblewski F., Colorimetric Measurement of Lactic Dehydrogenase Activity of Body Fluids, Am. J. Clin. Pathol., 30:234, 1958.

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